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Structural, thermal, and functional properties of *Agave tequilana* fructan fractions modified by acylation

Propiedades estructurales, térmicas y funcionales de fracciones de fructanos de *Agave tequilana* modificadas por acilación

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Abstract
Native Agave Fructans (NAF) are characterized by their high hydrophilicity, which limits their applications in food and other areas. In turn, the high content of OH groups in its structure allows its chemical modification easily. High Performance Agave Fructans fractions (HPAF) and a High Degree of Polymerization Agave Fructans (HDPAF) have been obtained through an ultradiafiltration. Therefore, the objective of the present study was to modify NAF and its fractions HPAF and HDPAF through acylation reaction with lauroyl chloride and to evaluate their physicochemical and functional properties at different pH levels. The characterization of the fructans fractions involved nuclear magnetic resonance (NMR), Fourier transforms infrared spectroscopy (FTIR) differential scanning calorimetry (DSC), and X-ray diffraction (XRD) techniques, which demonstrated the incorporation of the lauroyl functional group (chain of 12 carbon atoms) in the molecules, with degrees of substitution (DS) ranging from 2.03 to 2.36. The fractions showed significant changes in their functional (foaming, swelling, emulsification, and solubility, which depended on pH), physicochemical, and thermal properties. Therefore, this study confirmed that the acylation of NAF, HPAF, and HDPAF modifies their properties and provides an opportunity to diversify and expand their use in different areas.
Keywords: Esterification, functionality, chemical modification, amphiphilic molecules, biopolymer.

Resumen
Los Fructanos Nativos de Agave (FNA) se caracterizan por ser altamente hidrofílicos, lo que limita sus aplicaciones en alimentos y otras áreas. A su vez, el alto contenido de grupos OH en su estructura permite su modificación química fácilmente. Se han obtenido mediante ultradiafiltración, fracciones de Fructanos de Agave de Alto Rendimiento (FAAR) y Fructanos de Agave de Alto Grado de Polimerización (FAAGP). Por lo tanto, el objetivo del presente estudio fue modificar FNA y sus fracciones FAAR y FAAGP por reacción de acilación con cloruro de lauroilo y evaluar sus propiedades fisicoquímicas y funcionales a diferentes pH. La caracterización de las fracciones de fructanos involucró técnicas de resonancia magnética nuclear (RMN), espectroscopía infrarroja con transformada de Fourier (EITF), calorimetría diferencial de barrido (CDB) y difracción de rayos X (DRX), que demostraron la incorporación del grupo funcional lauroilo (cadena de 12 carbonos) en las moléculas, con grados de sustitución (GS) que oscilaron entre 2.03 y 2.36. Las fracciones mostraron cambios significativos en las propiedades funcionales (espumante, hinchamiento, emulsificación y solubilidad, que dependía del pH), fisicoquímicas y térmicas. Por lo tanto, este estudio confirmó que la acilación de FNA, FAAR y FAAGP modifica sus propiedades y brinda una oportunidad para diversificar y expandir su uso en diferentes áreas.
Palabras clave: Esterificación, funcionalidad, modificación química, moléculas anfifílicas, biopolímero.

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1 Introduction

Natural polymers, especially polysaccharides, possess fundamental qualities through their chemical structure that recommend their use in biomedical, food, beverages, pharmaceutical, cosmetic, personal care, and other purposes due to their biocompatibility, abundance, and functional properties. However, the growing demand for the development of biomaterials has imposed the need to modify the structure of these polymers to induce new properties and thus broaden the spectrum of applications and increase their value in the market (Desbrieres it et al., 2018). Modification through chemical reactions has been one of the most widely used techniques to modify their hydrophilic character by introducing acids with different degrees of hydrophobicity (Kontogiorgos, 2019). For polysaccharides with hydroxyl groups, esterification seems to be an excellent option to reduce hydrophilia (Choi *et al*., 2018), which may lead to improved functionality and new applications. It has been shown that larger hydrophobic groups, e.g., lauroyl or palmitoyl groups, can allow better encapsulation of hydrophobic drugs without harmful effects to the organism and exhibit antimicrobial action against Gram-positive bacteria (Li *et al*., 2021; Miramontes-Corona *et al*., 2020). Furthermore, several studies have shown that chemical modification has positive effects on various properties, expanding the fields of application of polysaccharides. In this context, one of the groups of biopolymers with the greatest potential is native agave fructans (NAF), which are a polydisperse mixture of sugars, fructo- and polysaccharides with 3-70 apparent degrees of polymerization (DPa), linked by glycosidic bonds (β 2-1) and (β 2-6), generating linear and highly branched structures that are synthesized in different agave species (Aldrete-Herrera *et al*., 2023; Mellado-Mojica and López, 2012). NAF possess technological and functional properties that benefit consumers by exhibiting their prebiotic and nutritional properties (García-Gamboa *et al*., 2020). Research studies have reported the ultradiafiltration of NAF to produce fractions with a reduction of simple sugars called High Performance Agave Fructans (HPAF) and High Degree of Polymerization Agave Fructans (HDPAF) (DPa >30) with different characteristics (Ceja-Medina *et al*., 2021; García-Gamboa *et al*., 2020; Aldrete-Herrera *et al*., 2023; Ortiz-Basurto *et al*., 2017). However, NAF and their fractions (HPAF and HDPAF), due to their composition and chemical structure, are characterized as highly soluble and hydrophilic compounds, which limits their application in some products. Therefore, NAF have been recently esterified with acetic and succinic anhydrides (Buitrago-Arias *et al*., 2021; Starbird *et al*., 2007) and different types of fatty acids (Miramontes-Corona *et al*., 2020, 2019) for the manufacture of drug delivery systems. Moreover, the acylation of NAF improves their technological properties (decreases solubility and increases emulsifying, foaming, and swelling capacity) (Ignot-Gutiérrez *et al*., 2020). Other authors have reported the cationization and carboxymethylation of agave fructans demonstrating a greater oil retention capacity and antimicrobial effect against Gram-negative bacteria (Castañeda-Salazar *et al*., 2023). So far, only one study has been found on the laurylated and succinylated HPAF and HDPAF fractions, which increase their antibacterial activity against pathogenic bacteria, while the acetylated fractions increase the prebiotic effect and favor the growth of *L. paracasei* (Díaz-Ramos *et al*., 2023). Therefore, the present study aimed to synthesize, characterize, and evaluate the physicochemical and functional properties at different pH of HPAF and HDPAF fractions chemically modified by the acylation process, since these fractions have aroused interest in being modified to obtain materials with better characteristics and applications.

2 Materials and methods

2.1 Materials

The Native Agave Fructans (NAF) Olifructine^{TM} were donated by Nutriagaves de México S.A. de C.V. The high-performance (HPAF) and high degree of polymerization (HDPAF) agave fructan fractions, were obtained according to Ceja-Medina *et al*., 2021. The distribution profile of the apparent degree of polymerization of all fructans was obtained, as reported by Aldrete-Herrera *et al*., 2023. The area under the curve was calculated by integrating the peak area (Mellado-Mojica and López, 2012) using the standards-based Chromeleon® software and inulin retention time to estimate the variations in the composition of the ultrafiltered fractions. NAF showed a maximum DPa of 70 (MWmax 12 KDa), with a 26% DPa concentration of 1-2; 24% fructooligosaccharides (FOS), 38% 10 to 30 and 13% DPa > 30, and the HDPAF fraction contained 9% DPa 1-2; 25% FOS, 32% from 10 to 30; and 34% DPa > 30. Among the physicochemical properties reported for these fructans are mainly moisture with values of 4.62, 3.0 and 2.52 (g 100 g^{-1}) for NAF, HPAF and HDPAF, respectively, and water activity (a_w) with 0.41. (NAF), 0.33 (HPAF), 0.3 (HDPAF) (Rodríguez-Furlán *et al*., 2014), among others shown in tables S1- S4. Lauroyl (C12) chloride (98% pure) was obtained from Sigma-Aldrich, USA.

2.2 Methods

2.2.1 Acylation of native fructans and fractions

Acylation was performed by adding 10 g of NAF, HPAF, and HDPAF, respectively, to a NaOH solution (50 mL, 20% w/v) at 25 °C under magnetic stirring at 300 rpm until complete dissolution. Subsequently, 3 mL of lauroyl chloride was added dropwise and the mixture was kept under magnetic stirring for 90 min. The resulting product was precipitated and filtered under a vacuum. Finally, the product was washed with water and subjected to thermal reflux for 8 h (by Soxhlet apparatus with hexane) to remove unreacted fatty acid chlorides and other byproducts. The product was then dried under a vacuum at 60 °C for 1 h and recovered as a solid white powder (Han *et al*., 2017).

2.2.2 Fourier Transform Infrared Spectroscopy-Attenuated Total Reflection (FTIR-ATR)

The IR spectra of unmodified and acylated NAF, HPAF, and HDPAF were analyzed by Fourier transform infrared spectroscopy using a Nicolet iS50 Thermo Scientific Spectrometer equipped with a diamond crystal attenuated total reflectance (Thermo Electron Scientific Instruments LLC, Madison, Wisconsin, USA). Samples (2 mg) were placed directly into the apparatus without prior preparation. FTIR-ATR spectra were recorded with a resolution of 8 cm^{-1} in the spectral range of 4000-400 cm^{-1}. Spectral analysis was performed with the OMNIC FTIR v9.0 software.

2.2.3 ^{1}HNMR Spectroscopy

^{1}HNMR spectra were acquired using a nuclear magnetic resonance spectrometer (DD_2 500 MHz, Agilent Technologies, Santa Clara, CA, USA) for both unmodified and acylated NAF, HPAF, and HDPAF. Fifteen mg of sample and 500 μL of D_2O were used as solvents in the unmodified fructans. Acylated $(CD_3)_2C{=}O$, CD_3OD, and $CDCl_3$ were used for NAF, HPAF, and HDPAF, respectively. The spectral analysis and visualization were performed using MestReNova software from Mestrelab Research, Chemistry Software Solutions (Version. 12.0.0-20080.2.2.3).

2.2.4 Degree of substitution

The degree of substitution (DS) of esterified NAF, HPAF and HDPAF was performed by calculating the ratio between the area under the peak at 0.8-1.2 ppm divided by three (assigned to the methylene protons) and the area under the signal of the glucosidic proton of glucose (Miramontes-Corona *et al*., 2020).

2.2.5 X-ray diffraction (XRD)

The powder X-ray diffraction patterns of both unmodified and acylated fructans were determined using a Bruker D_2 Phaser diffractometer (Bruker AXS GmbH, Karlsruhe, Baden-Württemberg, Germany) equipped with CuKα radiation (λ = 0.1541 A). The diffraction range used was 5-60° (2θ). The crystallinity index of the samples (x_c) was calculated using equation (1) (García-Barradas *et al*., 2022).

$$x_c = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (1)$$

Where I_{002} and I_{am} represent the peak intensities of crystalline and amorphous materials, respectively.

2.2.6 Physicochemical properties of unmodified and acylated fructans

The moisture content of both unmodified and acylated NAF, HPAF, and HDPAF was determined gravimetrically using a vacuum drying oven at 40 °C for 24 h until the samples reached constant weight (A.O.A.C). Water activity was measured at 25 °C using an Aqualab model Series 3 hygrometer, from Decagon Devices Inc, Pullman, WA, USA. The color of the samples was evaluated using a spectrophotometer (Hunter Lab Color Flex model CX115 45/0, Reston VA, USA) using the parameters L*, a*, and b* by the CIE Lab scale, and the Hue angle and Chroma were calculated.

2.2.7 Functional properties of unmodified and acylated fructans

2.2.7.1 Water Holding Capacity (WHC) and Oil Holding Capacity (OHC)

The water retention capacity of the fructan fractions was determined by adding 0.2 g of the sample to 2.5 mL of phosphate buffer pH 4, 7, and 10, respectively, in tubes. Likewise, for oil holding capacity, 0.2 g of the sample was added to 2.5 mL of pure soybean oil in tubes. The tubes were shaken in a vortex mixer (LP Vortex Mixer, Thermo Scientific) for 1 min (3000 rpm). Subsequently, the samples were allowed to rest for 30 min and centrifuged (Eppendorf brand, model 5804 R, Germany) at 1500 xg for 10 min. Finally, the water or oil released in the centrifugation was discarded, and the tubes were weighed. The water or oil retention capacity was expressed as grams of water or oil retained per gram of sample (Wani *et al*., 2013).

2.2.7.2 Swelling capacity (SC) and solubility index (SI)

The swelling capacity and solubility index were determined by placing 0.1 g of dry sample in centrifuge tubes, and 10 mL of phosphate buffer pH 4,

7, and 10, respectively. The mixture was heated at 55 °C for 30 min. After heating, the tubes were removed from the bath, allowed to cool, and centrifuged at 4032 xg for 20 min. The supernatant was transferred to previously tared aluminum capsules and heated until completely dry. The mass of the centrifuge tubes with the sediment and the mass of the capsules with the dried residue were recorded (Anderson, 1982). Equations 2 and 3 were used to calculate the solubility index and swelling capacity, respectively.

$$SI = \frac{\text{Solubles weight (g)}}{\text{Sample weight (g)}} \times 100 \quad (2)$$

$$SC = \frac{\text{Gel weight (g)}}{\text{Sample weight (g)-Solubles weight(g)}} \quad (3)$$

2.2.7.3 Emulsification capacity (EC)

For EC determination, 4% sample suspensions were prepared in phosphate buffer at pH 4, 7 and 10. To each suspension, 5 mL of soybean oil was added, and the mixture was homogenized at 100 rpm for 1 min with a homogenizer (model D-130, Wiggen Hauser Selangor, Malaysia). Finally, the samples were centrifuged (Centrifuge, Eppendorf brand, model 5804 R, Hamburg, Germany) at 1295 xg for 5 min at 20 °C. To calculate the percentage of emulsification, the following equation was applied.

$$EC = \frac{\text{Height of emulsionated layer}}{\text{Total height}} \times 100 \quad (4)$$

2.2.7.4 Foaming capacity

Foaming capacity was determined by preparing 2% w/v aqueous dispersion of the sample with phosphate buffer at pH 4, 7, and 10. The dispersions were homogenized at 10 000 rpm for 1 minute using a homogenizer (model D-130, Wiggen Hauser, Selangor, Malaysia). Foaming capacity was calculated as the percentage increase in the dispersion volume of the sample after foaming (Wani *et al*., 2013).

2.2.7.5 Thermal properties

The thermal properties of unmodified and acylated NAF, HPAF, and HDPAF were determined using a differential scanning calorimeter (DSC) (model Discovery, TA Instruments, New Castle, DE USA) in a nitrogen atmosphere. Each sample was heated from 10 to 200 °C at a rate of 5 °C/min (Ignot-Gutiérrez *et al*., 2020). An empty double capsule was used as a reference, and all determinations were performed in triplicate. The DSC equipment was preliminarily calibrated using a standard indium reference. For thermogravimetric analysis (TGA), the experimental conditions included a purge gas flow rate of 100 mL min^{-1}, and a heating rate of 10 °C min^{-1} with a temperature range from 10 to 300 °C. Alumina crucibles with samples of about 20 mg were used in the analysis.

2.2.7.6 Surface tension

Surface tension was measured using the Du Nouy ring method with a K8 tensiometer and a 4 cm circumference RI 01 platinum ring from Krüss GmbH. The concentrations used in each sample were 0, 0.05, 0.1, 0.15, 0.2, 0.25, mg/mL. All measurements were performed three times. The temperature was kept constant during the measurements at 25 ± 1 °C (Han *et al*., 2017).

2.2.7.7 Rheology

The viscosity of the apparent gel formed in acylated NAF, HPAF, and HDPAF was measured using a rheometer (AR-2000ex, TA Instrument Ltd, Leatherhead, England, USA) with a conical plate (2 cm diameter). The thickness of the sample was 1 mm. An increasing shear rate was applied, and measurements were made at 25 °C each.

2.2.7.8 Statistical analysis

The experiments were performed three times. The mean and standard deviation of the mean were calculated for each experimental parameter. Differences between experimental samples were determined by analysis of variance (one-way ANOVA). Pairwise multiple comparison tests (Holm-Sidak method) were applied, with a significance level of $\alpha < 0.05$. The statistical analysis was performed using the SigmaPlot statistical package version 12.0.

3 Results and discussion

3.1 Characterization of unmodified and acylated agave fructan fractions

3.1.1 FTIR characterization

The fractions of unmodified fructans and their acylated derivatives were analyzed by FTIR (Figure 1). In the unmodified samples, stretching bands at 3268 and 3274 cm^{-1} were observed, which are characteristic of -OH groups for NAF, HPAF, and HDPAF. Additionally, bands at 1032 and 1012 cm^{-1} were observed, indicating the presence of C-O-C groups in the fructans. In the acylated fructans, new absorption bands appeared at 2921 and 2847 cm^{-1} due to the symmetric and asymmetric stretching of the CH_3-CH_2 bonds of the incorporated lauroyl chains. Another significant band was observed at 1557 cm^{-1}, which indicates the presence of the carbonyl group, corresponding to the formation of esters in

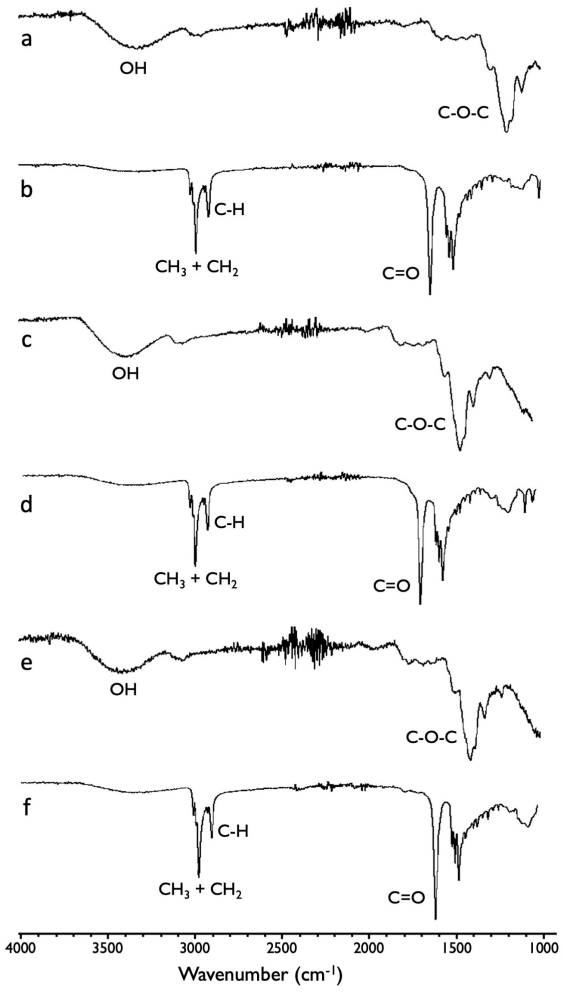

Figure 1. FTIR spectra of agave fructans, (a) NAF, (b) acylated NAF, (c) HPAF, (d) acylated HPAF, (e) HDPAF, (f) acylated HDPAF.

the fructans. This shift in the absorption band could be attributed to H-bond rearrangements and different nonpolar interactions between the fatty acid chains (Taresco *et al*., 2016). It has been reported that O- and N-bonded acyl groups can be distinguished by the absorption of C-O and C-N vibration around 1245 and 1550 cm^{-1}, respectively (da silva *et al*., 2021; Shi *et al*., 2017). The above agrees with the results obtained in other studies (Ignot-Gutiérrez *et al*., 2020) on acylated NAF, that have reported the C=O band at 1556 cm^{-1}. The decrease in the intensity of the -OH band observed with increasing acyl groups is associated with the degree of substitution, indicating a successful acylation reaction. Similar results have been reported for agave fructans (Miramontes-Corona *et al*., 2020, 2019) and modified inulins (Peng *et al*., 2019; Petkova *et al*., 2017). The differences observed in the studies consulted could be related to the degree of polymerization, the agave species, and the type of fructan used (linear or branched).

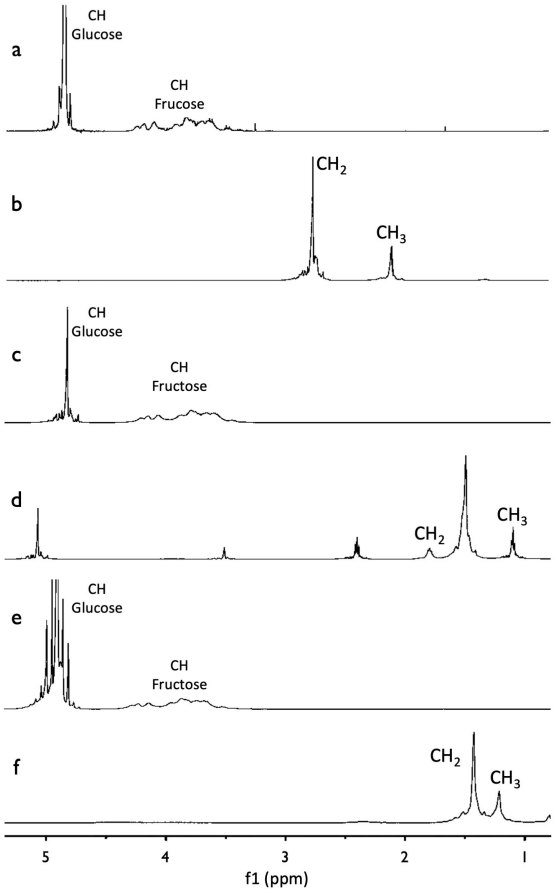

Figure 2. ^{1}H NMR spectra of agave fructans, (a) NAF, (b) acylated NAF, (c) HPAF, (d) acylated HPAF, (e) HDPAF, (f) acylated HDPAF.

3.1.2 ^{1}H NMR characterization

Figure 2 shows the ^{1}H NMR spectra of the unmodified and acylated fructans. Predominant peaks were observed at 4.66, 4.64, and 4.67 ppm, corresponding to the anomeric glucose proton. At 3.65, 3.72, and 3.73 ppm the fructose proton signal was found to be present for NAF, HPAF, and HDPAF, respectively. The acylated fructans for NAF showed signals at 2.05 and 2.65 ppm; those for HPAF at 0.89, 1.28, and 2.19 ppm, and those for HDPAF at 1.28 and 1.47 ppm. These signals are characteristic of the methyl and methylene groups of the incorporated fatty acid chain, demonstrating the modification of the fructans. These results are similar to those reported for NAF, with signals at 4.5-3.1 ppm for fructose and at 0.86 and 1.12 ppm corresponding to methyl and methylene groups of the modified fructans (Miramontes-Corona *et al*., 2019). The acylated samples showed degrees of substitution (DS) of 2.36, 2.03, and 2.30 for NAF, HPAF, and HDPAF, respectively. Other studies (Castañeda-Salazar *et al*., 2023; Miramontes-Corona *et al*., 2019) have reported lower DS in agave fructans than in the present work; these differences are attributed to factors such as DP and the method used.

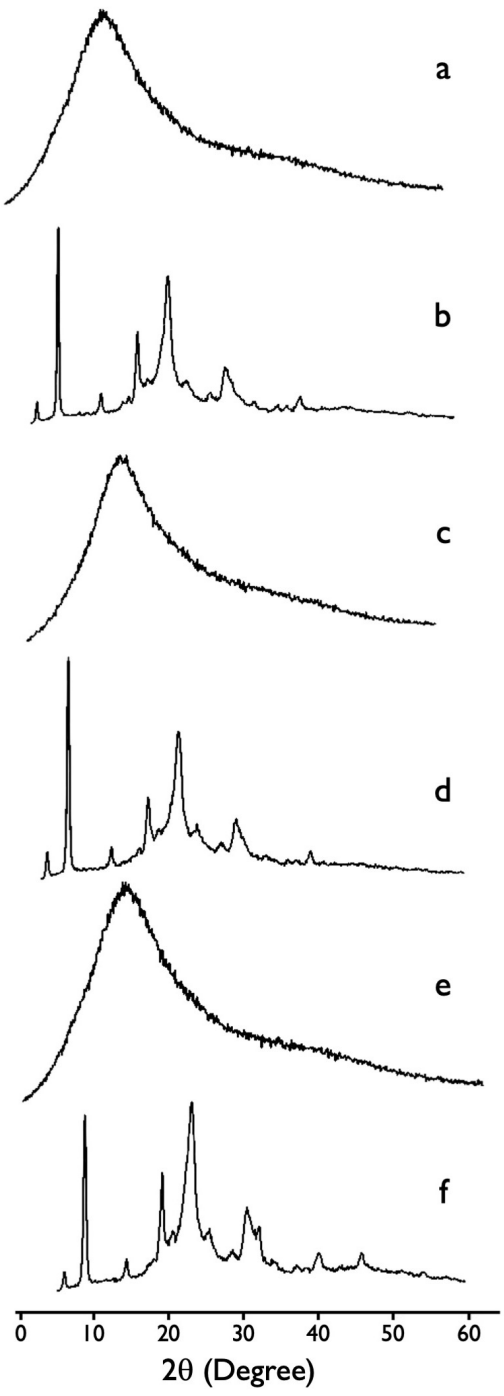

Figure 3. Diffractograms of agave fructans, (a) NAF, (b) acylated NAF, (c) HPAF, (d) acylated HPAF, (e) HDPAF, (f) acylated HDPAF.

3.1.3 X-ray diffraction characterization

X-ray diffraction spectroscopy confirmed the changes in the structure of the acylated fructans at the molecular level. Figure 3 shows the X-ray diffraction patterns of the unmodified and acylated fructans. It is observed that the unmodified fructans exhibit an amorphous structure due to the presence of a broad peak ranging from 2θ = 10° to 29°. This peak is attributed to the high polarity of agave fructans, since they are soluble in water and therefore present amorphous structures due to the high degree of molecular branching (Fernández-Lainez *et al*., 2021). In the acylated fructans, patterns of semicrystalline arrangements are observed in the X-ray diffraction patterns, i.e., some thin and long peaks indicate the presence of dispersion patterns characteristic of crystalline structures according to their position and intensity (Toda, 2020), while the broader peaks correspond to amorphous structures. Therefore, the incorporation of lauroyl chains in acylated fructans leads to a semicrystalline structure, altering their polarity. It has been reported that physical and chemical modifications applied to polysaccharides lead to significant changes in the degree of polysaccharide crystallinity by manipulating intramolecular interactions (Farzad *et al*., 2021). The most intense peaks observed in the diffractograms of acylated fructans were found at the 2θ value of around 8.55° in NAF and HPAF, and 8.73° in HDPAF, indicating the crystal structure in acylated fructans. The higher and sharper the diffraction peak, the higher the degree of crystallinity of a biopolymer due to the removal of amorphous regions (Baraka *et al*., 2023). The acylated fructans presented crystallinity indices of 35.59%, 37.51%, and 43.35% for NAF, HPAF, and HDPAF, respectively, being slightly higher than those given in the unmodified ones with indices of 34.76% (NAF), 34.46% (HPAF) and 23.88% (HDPAF). However, so far no more studies have been found where the crystallinity index of these fractions in agave fructans is reported. The degree of crystallinity was higher for HDPAF, possibly because the amount of amorphous material in these samples was reduced during the acquisition process. The differences observed in the diffractograms, as well as in the degree of crystallinity between NAF, HPAF, and HDPAF, are attributed to the DP since it has been reported that it is a factor that influences the structure and changes of the polysaccharides, affecting their flexibility or rigidity, which depends on the geometry of the glycosidic bond (Mansel *et al*., 2020). This is similar to what was found in the present study, where it was shown that the degree of crystallinity increases when the DP is higher in fructans. Furthermore, it has been reported that the differences in the crystallinity index can be caused by intermolecular bonds formation resulting from the processing methods to which the biopolymers are subjected (Zapata-Luna *et al*., 2023).

Table 1. Physicochemical properties of unmodified and acylated (AC) native agave fructans (NAF), high performance agave fructans (HPAF) and high degree of polymerization (HDPAF).

Property	NAF	NAF_{AC}	HPAF	$HPAF_{AC}$	HDPAF	$HDPAF_{AC}$
Moisture content (g H_2O /100 g)	5.09 ± 0.55^d	6.45 ± 1.17^e	2.95 ± 0.57^a	3.42 ± 1.06^c	2.64 ± 0.64^b	3.57 ± 0.87^c
Water activity (25°C)	0.22 ± 0.01^c	0.10 ± 0.03^b	0.21 ± 0.01^c	0.31 ± 0.01^d	0.08 ± 0.01^a	0.10 ± 0.02^b
Color parameters						
L^*	94.92 ± 0.27^e	95.26 ± 0.04^f	85.64 ± 1.71^a	92.44 ± 0.10^b	94.59 ± 0.07^d	93.09 ± 0.03^c
a^*	-0.57 ± 0.36^b	-0.86 ± 0.11^d	-0.68 ± 0.06^c	-0.08 ± 0.11^a	-0.94 ± 0.09^f	-0.92 ± 0.04^e
b^*	2.27 ± 0.47^a	2.89 ± 0.08^d	3.9 ± 0.12^e	2.21 ± 0.24^a	2.37 ± 0.13^b	2.78 ± 0.02^c
Hue (°)	-76.47 ± 5.42^e	-73.39 ± 1.8^d	-79.93 ± 0.52^f	-70.09 ± 0.6^c	-67.19 ± 2.58^a	-71.56 ± 0.71^b
Chroma	2.34 ± 0.54^a	3.01 ± 0.10^d	3.99 ± 0.11^e	2.35 ± 0.27^a	2.54 ± 0.15^b	2.92 ± 0.04^c
Browning index	4.46 ± 0.23^c	4.99 ± 0.11^d	3.95 ± 0.02^a	4.28 ± 0.18^b	4.29 ± 0.23^b	4.89 ± 0.30^d

The data represent the mean of 3 replicates ± standard deviation. Different letters between columns indicate a significant difference ($p < 0.05$) between unmodified and acylated fructans.

3.2 Physicochemical properties of unmodified and acylated agave fructan fractions

Table 1 shows the effect of modification on the physicochemical properties of the fructans. The unmodified and acylated NAF presented the highest moisture content, with significant differences (P < 0.05) between the NAF and the unmodified and acylated fractions. These differences could be due to the content of free sugars and the DP, as well as to the substitution of OH groups, which reduce their hygroscopicity (Ignot-Gutiérrez *et al*., 2020). Concerning water activity, unmodified fructans presented values ranging from 0.08 to 0.22, while the acylated fructans showed a range from 0.10 to 0.31. These values indicate that both unmodified and acylated fructans are suitable for inhibiting microbial growth as well as reducing biochemical reactions (Tapia *et al*., 2020). The increase of moisture and water activity in acylated fructans could be due to the affinity and binding forces between water and the fructan surface. The introduction of lauroyl chains (hydrophobic groups) results in an increase in the frequency of charges and electronegativities resulting from acylation (Yin *et al*., 2010), causing electrostatic repulsion. In other words, the hydrophobic chains significantly reduce the molecule´s polarity, causing lower solubility in water and higher solubility in organic solvents. This repulsion could decrease the interaction of water (Zhao *et al*., 2017) and increase the exposure of water on the fructan surface (hydrophobic hydration). Therefore, by reducing the interaction with water molecules by increasing lipophilicity, free water, which has low mobility, decreases (Chakravarty and Nagapudi, 2021). Consequently, the risk of altering the properties of the system is reduced.

Color measurement is important as a physical and quality assessment, as it can be affected by various factors (Mathias-Rettig and Ah-Hen, 2014). CIE L*, a*, b* color parameters of unmodified and acylated fructans were evaluated. Both in luminosity and in a* and b*, there are differences ($p < 0.05$) between the unmodified and acylated fructans. The increase in lightness can mean that the fructans increase their brightness and, in turn, modify the values of a* and b*. However, these variations did not change the color of the fructans (Figure S2); on the contrary, they visibly improved by being brighter, which is attractive to be incorporated into any product. Regarding the browning index, the differences presented ($p < 0.05$) were minimal. These variations are attributed to the change in the structure of the fructans given by the fatty acid (Ignot-Gutiérrez *et al*., 2020).

3.3 Functional properties of unmodified and acylated fructans

The introduction of a new functional group caused significant changes in the polarity of each of the samples ($p < 0.05$). This is attributed to the insertion of lauroyl chloride converting the hydroxyl (OH) groups of the fructan units into hydrophobic esters, hindering the interaction with water molecules, which modifies the solubility and hydrophobicity of the polysaccharide (Salgado-Delgado *et al*., 2022). Table 2 presents the functional properties evaluated in the unmodified and acylated agave fructans at three different pH. The decrease in solubility, in turn, caused a change in the water-holding capacity (WHC), it being slightly higher at pH 10. This can be attributed to the fact that there is a higher equilibrium or saturation at alkaline pH, so there is an increase in water absorption at the surface of the molecule (Ramaswamy *et al*., 2013). Additionally, the incorporation of fatty acid chains in the fructan structure produces a distribution of hydrogen bridges, steric hindrance, and inter- and intraparticle disorganization that favors water access in the amorphous region (Ignot-Gutiérrez *et al*., 2020).

Table 2. Functional properties at different pHs of unmodified and acylated (AC) native agave fructans (NAF), high performance acylated agave fructans fractions (HPAF) and high degree of polymerization (HDPAF).

Property	pH	NAF	NAF_{AC}	HPAF	$HPAF_{AC}$	HDPAF	$HDPAF_{AC}$
Solubility index (%)							
	4	$100 \pm 0.01^{c,A}$	$19.62 \pm 0.65^{b,B}$	$100 \pm 0.01^{c,A}$	$20.34 \pm 2.63^{b,C}$	$100 \pm 0.01^{c,A}$	$32.96 \pm 0.98^{a,B}$
	7	$100 \pm 0.01^{d,A}$	$26.96 \pm 2.46^{c,A}$	$100 \pm 0.01^{d,A}$	$86.90 \pm 0.30^{a,A}$	$100 \pm 0.01^{d,A}$	$49.90 \pm 4.02^{b,A}$
	10	$100 \pm 0.01^{c,A}$	$5.45 \pm 2.79^{b,C}$	$100 \pm 0.01^{c,A}$	$22.09 \pm 0.46^{a,B}$	$100 \pm 0.01^{c,A}$	$5.24 \pm 0.66^{b,C}$
Water holding capacity	4	$0.01 \pm 0.01^{a,A}$	$3.24 \pm 0.31^{d,A}$	$0.01 \pm 0.01^{a,A}$	$2.58 \pm 0.29^{b,A}$	$0.01 \pm 0.01^{a,A}$	$2.98 \pm 0.08^{c,B}$
(g water/g sample)	7	$0.01 \pm 0.01^{a,A}$	$3.58 \pm 0.07^{c,A}$	$0.01 \pm 0.01^{a,A}$	$3.36 \pm 1.09^{d,B}$	$0.01 \pm 0.01^{a,A}$	$3.04 \pm 0.01^{b,B}$
	10	$0.02 \pm 0.02^{a,A}$	$5.75 \pm 0.08^{d,B}$	$0.01 \pm 0.01^{a,A}$	$4.98 \pm 0.16^{c,C}$	$0.02 \pm 0.01^{a,A}$	$2.21 \pm 0.04^{b,A}$
Foaming capacity (%)							
	4	NP	$120 \pm 0.01^{a,A}$	NP	$169 \pm 1.41^{b,A}$	NP	$120 \pm 0.01^{a,A}$
	7	NP	$390 \pm 14.14^{b,B}$	NP	$290 \pm 14.14^{a,C}$	NP	$290 \pm 14.14^{a,B}$
	10	NP	$580 \pm 28.28^{e,C}$	NP	$220 \pm 28.28^{c,B}$	NP	$420 \pm 28.28^{d,C}$
Swelling capacity							
(mL/g)	4	$0.10 \pm 0.01^{a,A}$	$32.20 \pm 3.10^{c,A}$	$0.17 \pm 0.01^{a,A}$	$51.82 \pm 2.88^{d,B}$	$0.10 \pm 0.01^{a,A}$	$25.95 \pm 4.06^{b,A}$
	7	$0.10 \pm 0.01^{a,A}$	$35.76 \pm 0.94^{d,B}$	$0.10 \pm 0.01^{a,A}$	$32.76 \pm 1.36^{c,A}$	$0.10 \pm 0.01^{a,A}$	$30.24 \pm 0.16^{b,B}$
	10	$0.10 \pm 0.01^{a,A}$	$94.54 \pm 2.79^{c,C}$	$0.10 \pm 0.01^{a,A}$	$77.90 \pm 0.46^{b,C}$	$0.10 \pm 0.01^{a,A}$	$94.57 \pm 0.66^{c,C}$
Emulsifying capacity							
(%)	4	NP	$18.16 \pm 0.23^{b,A}$	NP	$29.58 \pm 0.58^{c,B}$	NP	$14.83 \pm 0.23^{a,A}$
	7	NP	$29.66.\pm 0.47^{b,C}$	NP	$30.83 \pm 1.17^{c,C}$	NP	$27.75 \pm 0.11^{a,C}$
	10	NP	$26.41 \pm 0.35^{c,B}$	NP	$25.58 \pm 0.82^{b,A}$	NP	$24.83 \pm 0.23^{a,B}$
Oil holding capacity							
(g oil/g sample)		0.12 ± 0.01^{a}	10.19 ± 0.35^{c}	0.18 ± 0.0^{a}	8.26 ± 0.15^{b}	0.10 ± 0.01^{a}	10.78 ± 0.77^{c}

Different lowercase letters between columns indicate differences ($p < 0.05$) between unmodified and acylated fructans. Different capital letters between rows indicate differences ($p < 0.05$) between each pH. *Not present (NP).

Unmodified fructans showed a low oil retention capacity compared to acylated fructans, which is related to their chemical composition since the molecular arrangement caused by lauroyl chains promotes intermolecular interactions that increase the chemical affinity of fructan with the oil (Castañeda-Salazar *et al*., 2023). The unmodified fructans did not show emulsifying capacity, whereas the acylated fructans showed values ranging from 14 to 30%. This change can be attributed to the increase in DS and the length of the hydrocarbon chain that improves the emulsifying properties (Zhang *et al*., 2023).

The swelling capacity of a molecule depends on the acidic or basic groups present in its structure, such as -OH, -COOH, and -$CONH_2$, among others. Acylated fructans exhibited higher values of swelling capacity (30-94 mL/g) at pH 10 compared to unmodified samples (0.10-0.17 mL/g). This increase could be related to the ionization of the hydrophilic groups still present in the acylated fructans, which increase their ionization strength in the basic medium, causing an electrostatic repulsion that leads to the expansion of the polymeric network (Chandrika *et al*., 2014).

On the other hand, the modified fructans exhibited high foaming capacity values of 120-580%. In contrast, the unmodified fructans did not present this property, suggesting the potential use of acylated fructans as thickening agents and stabilizers, among other related applications (García-Barradas *et al*., 2022; Hernández-Pérez *et al*., 2022).

3.4 Thermal analysis of fructans

The thermal properties of the fructans were evaluated by differential scanning calorimetry (DSC) both before and after chemical modification. Figure 4 shows the changes observed before and after acylation. In acylated fructans, different thermal transitions are observed between each fructan, being more noticeable in NAF and HPAF. This could have been caused by possible interactions between hydrophilic and hydrophobic segments (Taresco *et al*., 2016). Polymers can present various conversions and new properties by acylation. In this sense, changes in thermal transitions observed in acylated fructans could be due to a significant effect caused by long carbon chains in the polar intermolecular interactions of the main polymer backbone (Kunisada *et al*., 1990). In the thermograms of acylated fructans, endothermic and exothermic peaks are observed, which translate to melting and crystallization transitions, respectively. Therefore, the presence of these peaks highlights two separate phases, one amorphous and the other crystalline, which is attributed to the characteristic behavior of semicrystalline polymers. In addition, it has been reported that endothermic signals are related to the incorporation of fatty acids (Taresco *et al*., 2016), which is congruent with what was obtained in the present work (Figure 4). The above is similar to what was observed in the diffractograms (Figure 3), where the acylated fructans present a semicrystalline order structure. The signal shifts between acylated fructans could be due to the DP, molecular weight, and DS (Ignot-Gutiérrez *et al*., 2020; Espinoza-Andrews and Urías-Silvas, 2012;).

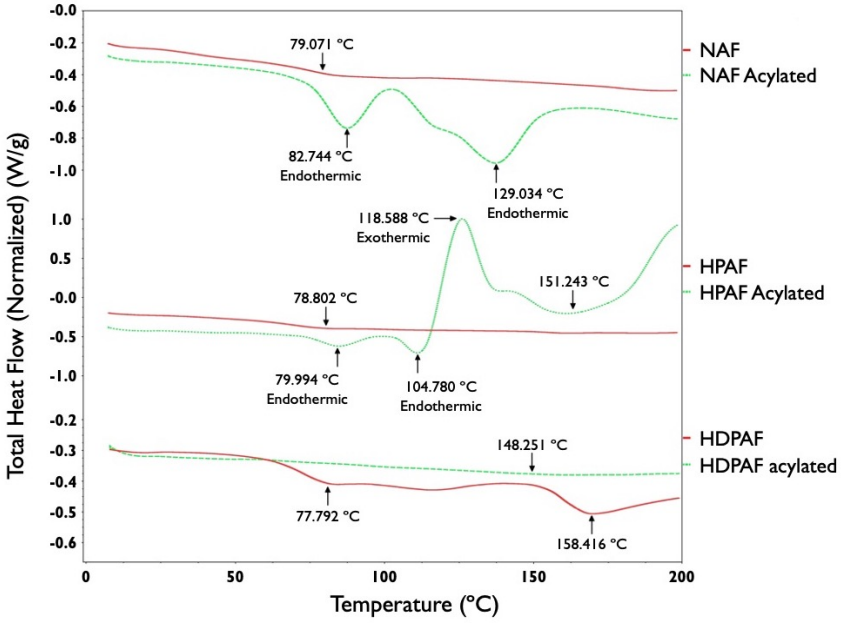

Figure 4. Thermograms DSC of agave fructans and their fractions before and after acylation.

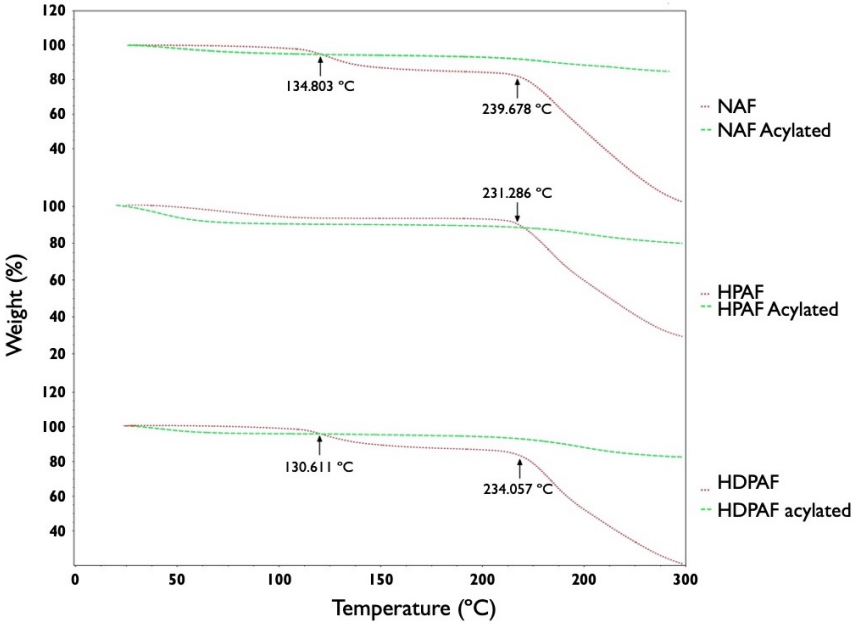

Figure 5. Thermograms TGA of agave fructans and their fractions before and after acylation.

In unmodified agave fructans, no crystallization peaks are observed due to their branched nature (Miramontes-Corona *et al*., 2020), resulting in amorphous structures. The thermal properties of the HDPAF fraction were less affected after the modification compared to NAF and HPAF. This difference is possibly attributed to the DP and the total or partial reduction of simple sugars (Ortiz-Basurto *et al*., 2017).

Regarding the thermogravimetric analysis (TGA), Figure 5 shows the weight loss as a function of increasing the temperature for each fructan. It is observed that the unmodified fructans present the most significant weight loss compared to the acylated ones. A change in two main stages is observed in the unmodified fructans, the first at around 130 °C with a loss of about 5% and the second at 230 °C with a loss of around 20%. This could be related to the endothermic peaks observed in the thermogram obtained by DSC. The weight loss of the first stage could be attributed to the dehydration process, while that of the second stage could be due to decomposition processes and caramelization, among other consecutive reactions, until reaching the

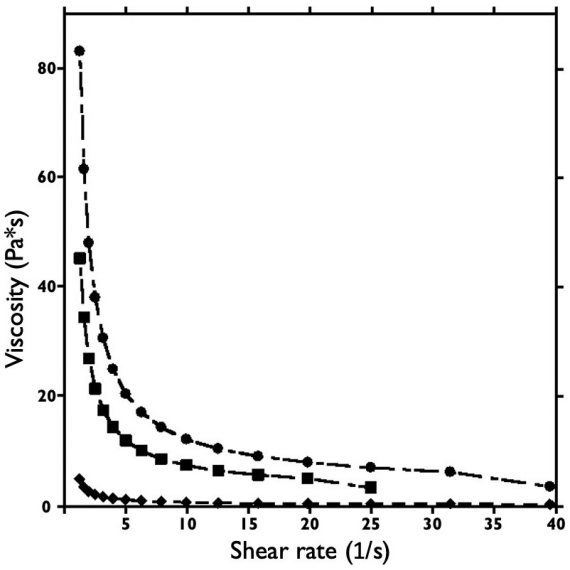

Figure 6. Viscosity curves of acylated agave fructans, (●) NAF, (■) HPAF, (♦) HDPAF at pH 10.

decomposition of organic matter (Leone *et al*., 2014). In contrast, the acylated fructans remain stable up to 300 °C, having weight loss of approximately 5-10%, with similar behavior between the acylated NAF and the acylated HPAF and HDPAF fractions.

3.5 Viscosity

Figure 6 shows the behavior of the viscosity of the gel formed in the acylated fructans at pH 10. It is observed that NAF presented the highest viscosity, followed by HPAF, whose viscosity decreases as the shear rate increases, presenting a shear-thinning behavior (Ceja-Medina *et al*., 2020). This behavior reflects the breakdown of the internal structure of the fluid as the molecular interactions weaken and the particles are oriented along the flow direction (Cervantes-Martínez *et al*., 2014). The formation of an apparent gel (Figure S1) is attributed to the lauroyl chains incorporated into the fructans, which modifies their structure and, consequently, their rheological behavior. This phenomenon is in contrast to unmodified agave fructans, which do not form gels due to their branched structure, maintaining a more significant interaction with water (Rodriguez-González *et al*., 2019). This is consistent with what has been reported for other types of biopolymers, where the introduction of the acylated compound demonstrates linear viscoelastic gel-like behavior in oscillating shear measurements, as well as higher thermal resistance with the inclusion of acyl groups (Sánchez *et al*., 2015). Likewise, it has recently been reported that polysaccharide modification increases viscosity and is related to DS, which can be attributed to the association between hydrophobic molecules and the formation of multimolecular clusters (Gahruie *et al*., 2020; Maedeh *et al*., 2020). On the other hand, HDPAF showed viscosity values close to zero, which may be attributed to the high DP causing the formation of a weaker gel (Sánchez *et al*., 2015).

3.6 Surface tension

The surface tension of the acylated fructans 55.13, 59.30, and 57.50 mN/m decreased compared to the unmodified fructans: 67.18, 63.54, and 67.20 mN/m for NAF, HPAF, and HDPAF, respectively (Figure 7). This decrease may be attributed to the addition of hydrophobic groups to their structure, as the unmodified fructans possess higher surface activity due to being highly hydrophilic (Maedeh *et al*., 2020). It was also observed that the acylated fructans at concentrations of 0.05 mg/mL in NAF, 0.05-0.01 mg/mL in HPAF, and 0.2-0.25 mg/mL in HDPAF had no significant changes in surface tension; this is considered the critical concentration (Flores-Andrade *et al*., 2021) because a micelle is formed at the hydrophobic ends of the fatty acid and the hydrophilic ends of the fructose, which protect the formed micelle from external influences by repulsive forces (Han *et al*., 2017). The differences in the behavior of the surface tension between the NAF and the fractions could be due to their molecular size and their DS.

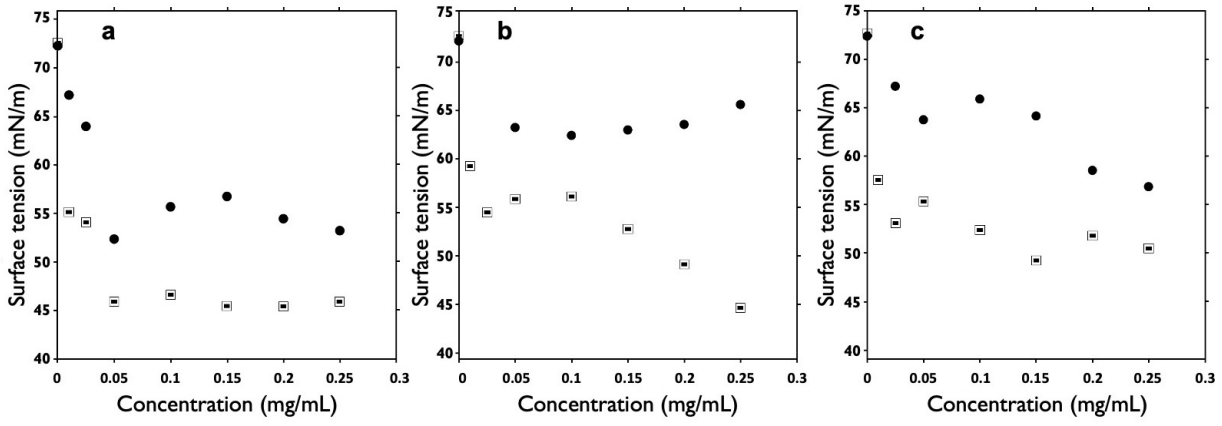

Figure 7. Surface tension of agave fructans: (a) NAF, (b) HPAF, (c) HDPAF, (•) unmodified and (□) acylated at different concentrations.

As more lauroyl chains are substituted in each fructan, the molecule becomes less polar. This results in fewer molecules of water being exposed in the vapor phase, leading to an increase in the surface tension of the chains and, on the other hand, those of water decrease (Hantal *et al*., 2019).

Conclusions

The present study reveals that native agave fructans and their high-performance, high-polymerization fractions can be modified by acylation with a lauroyl group, with a high degree of substitution. This chemical modification was confirmed by FTIR, NMR, XRD and DSC, demonstrating that the modification produced changes in its physicochemical and functional properties at the evaluated pH. This modification led to a decrease in solubility and an increase in foaming capacity and swelling capacity at pH 10, which allowed the formation of a type of gel, which can be used in the administration of specific drugs. The fractions of agave fructans modified by acylation showed greater thermal stability than the unmodified compounds. The results obtained in this study lay the foundations for developing new products based on agave fructans and their modified fractions, becoming partially hydrophobic agave fructans, and proposing applications as emulsifiers or additives in food, beverages, pharmaceutical, cosmetic and personal care products, among others.

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